

**EVALUATION OF THE MINNESOTA VOLUNTARY JOHNE'S DISEASE  
HERD STATUS PROGRAM FOR CATTLE AS A CONTROL MEASURE FOR  
PREVENTION OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS*  
INFECTIONS IN HUMANS.**

Daniel Arthur Kovich

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## ABSTRACT

The objectives of this study were to evaluate the perceived value enrolled producers gained from participation in the Voluntary Johne's Disease Herd Status Program (VJDHSP), and the risk of infection with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) of cattle reared in a presumed Johne's free environment versus cattle raised in an environment of unknown Johne's status. Producers enrolled in Level 3 or 4 of the VJDHSP were interviewed via phone survey. Producers were asked questions pertaining to their participation in the VJDHSP, and asked to identify herds to which they had sold replacement heifers. Prior to sale, these cattle were presumed to be uninfected. 59 cows were identified as having been raised in uninfected herds and sold to producers with herds of unknown Johne's disease status. On the purchasing farm, fecal and blood samples were taken from each cow of VJDHSP origin and three randomly selected home-reared cows per VJDHSP cow matched by lactation as controls. Samples were tested using commercial ELISA (serum) and bacterial culture (feces). Results indicated that enrolled producers saw value in VJDHSP participation, and that cattle reared in VJDHSP herds were less likely to be infected with MAP than herdmates as measured by serum ELISA MAP antibody and by fecal culture. This study provides evidence of the value of the VJDHSP in providing economic value to participants and supports the promotion of VJDHSP herds as a source of replacement cattle of low infection risk for MAP. Concern over the potential link between Johne's disease and Crohn's disease in people makes this validation valuable in attracting dairy producers to this potential control strategy.

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**Introduction**

Johne’s disease in cattle is a significant cause of economic loss to the dairy industry, and its relationship with Crohn’s disease in humans has caused significant debate. The diseases have many clinical and pathological similarities. Evidence exists to support an association between *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the causative agent of Johne’s disease, and Crohn’s disease. Human exposure to MAP can occur through direct, environmental, and dietary routes. Control of MAP infection in cattle would affect a substantial reduction in these known exposure categories. Should an association between Johne’s disease and Crohn’s disease be conclusively established, control may be mandated. This study seeks to validate the efficacy of one part of the current US control program, the Voluntary Johne’s Disease Herd Status Program (VJDHSP) for cattle (currently called the Johne’s Disease Test Negative Program), as a strategy for limiting human exposure to MAP.

**An Overview of Johne’s Disease**

Johne’s disease (also known as paratuberculosis) is a debilitating diarrheal disease of ruminants caused by MAP. MAP infects the M cells of the ileum, and leads to a gradual thickening of the intestinal mucosa and inability to absorb nutrients. In cattle,

clinical signs include progressive diarrhea and weight loss leading to death. Infected cattle are capable of shedding the organism in their milk, feces, and uterine secretions. The disease is predominantly spread via the fecal-oral route. The disease has a long period of incubation, and clinical signs are rarely seen before two years of age (Whitlock & Buergelt, 1996). Resistance to infection is believed to increase with age. This age-related resistance forms the basis of most control programs, which focus on rearing calves in an environment free from Johne's disease (Goodger et al., 1996). Johne's disease has a high prevalence in the United States. Nationally, at least 22% of all dairy herds and 40% of those with 300 or more cows are believed to be infected. The disease can cost farmers production losses of \$200 per cow in inventory in heavily infected herds, and the total cost to the US dairy industry is in excess of \$200 million annually (Ott, Wells, & Wagner, 1999).

### **An Overview of Crohn's Disease**

Crohn's disease is a chronic disease of humans that causes inflammation of the gastrointestinal tract. Though it can occur anywhere in the digestive tract, it has an affinity for the ileum. Inflammation of the intestinal mucosa frequently results in pain and diarrhea for the Crohn's sufferer. The etiology of Crohn's disease is unknown, but autoimmune activity and MAP are two of the most commonly postulated contenders. The Centers for Disease Control estimates the prevalence of Crohn's disease in the United States at 0.15 %, or 150/100,000 (Ashford, Imhoff, & Angulo, 2001). Crohn's disease is diagnosed most frequently in people between the ages of 20 and 30, and displays no gender bias (Sandler & Golden, 1986). Approximately 20 % of Crohn's

disease patients have a relative with the disease (Freeman, 2002). Though Crohn's disease is classified as chronic, treatment options including drug therapy, nutritional supplementation, and surgery have been shown to be effective in alleviating clinical manifestations of disease (Panaccione & Sandborn, 2004).

### **The Johne's Disease/Crohn's Disease Link**

There is a long history of scientific debate about the potential link between Johne's disease and Crohn's disease. To date, there is no consensus in the literature regarding this issue, and contradictory reports continue to be published. There have been three recent thorough reviews of the association—by the National Institute of Allergy and Infectious Diseases (1998), the European Commission (2000), and the National Research Council (2003). All conclude that though a definitive link has not been found, enough evidence exists to warrant further research. Following is a discussion of studies conducted after the National Research Council (NRC) report and their relationship to its findings.

Reports of Crohn's disease clusters are rare in medical literature. It is significant that since the publication of the NRC report, three new studies regarding Crohn's disease clusters have been released and all offer support to a link between Crohn's disease and MAP. A 2005 study found evidence of association between a cluster of Crohn's disease patients in Cardiff, Wales and MAP contamination of a local waterway (Pickup et al., 2005). Untreated water and consumption of unpasteurized cheese were implicated as risk factors in a study of family clusters in Belgium (Van Kruiningen et al., 2005). A study of

sibling Crohn's disease clusters suggested that environmental as well as genetic factors contribute to familial Crohn's disease clusters (Hugot et al., 2003).

The NRC report lists several categories of human studies that are directed at the association between Crohn's disease and MAP. Nine studies are reported concerning culture of MAP from tissue samples taken from Crohn's disease patients. Since publication of the NRC report, a new study has corroborated the ability to culture MAP from the blood of Crohn's disease patients—MAP was cultured from the blood of 50% of Crohn's disease patients and zero % of controls (Naser, Ghobrial, Romero, & Valentine, 2004).

The NRC report lists 16 studies regarding PCR identification of MAP DNA in tissue from Crohn's disease patients. New research in this area is conflicting. One additional study found significantly higher prevalence of MAP DNA in intestinal tissue from Crohn's disease patients (87%) compared to 17% in non-infected controls (Romero, Hamdi, Valentine, & Naser, 2005). This is in stark contrast to another study which found zero % prevalence of MAP DNA in Crohn's disease patient intestinal samples versus 32 % prevalence in samples from healthy controls (Bernstein, Nayar, Hamel, & Blanchard, 2003). In an additional study, MAP DNA was detected via PCR in 69 % of tissue samples from known Crohn's disease patients (Sechi et al., 2004). Another study utilized PCR to confirm MAP strain similarity between human and animal MAP infections (Ghadiali, Strother, Naser, Manning, & Sreevatsan, 2004).

Another major category of human studies concerning MAP focuses on serum antibody response to MAP antigens, and the NRC report lists seven pertinent studies. A new study has been published regarding seropositivity rates in Manitoba, Canada

(Bernstein, Blanchard, Rawsthorne, & Collins, 2004). This study found that all groups studied—Crohn’s disease patients, ulcerative colitis patients, unaffected siblings of both patient groups, and healthy controls—were approximately 35 % seropositive for MAP. Though this does not seem to offer evidence supporting a potential link between MAP and Crohn’s disease, it must be evaluated in the context that these seropositivity rates are significantly higher than those reported elsewhere, and that Manitoba has been reported to have the highest incidence of Crohn’s disease of any studied geographic population worldwide (Bernstein, Blanchard, Rawsthorne, & Wajda, 1999).

The NRC report concludes by using both Koch’s postulates and the Hill-Evans criteria to evaluate a cause-and-effect relationship between MAP and Crohn’s disease. It concludes that all four of Koch’s postulates are at least partially fulfilled, providing enough evidence to support an association. There is no new evidence to discredit this interpretation. The NRC report concludes that data is either insufficient, conflicting, or inconclusive for all ten of the Hill-Evans criteria, and new evidence does not change this conclusion. More research is needed to satisfy these criteria, leaving the question of a Johne’s disease/Crohn’s disease link open.

### **Human Exposure to MAP**

Human exposure to and infection with MAP is known to occur, and in some cases has been linked to Crohn’s disease in humans. Though human-to-human transmission is theoretically possible, most known exposure routes are from animals. These include direct exposure to infected animals or diagnostic samples, environmental exposure primarily via contaminated water, and dietary exposure via milk or meat.

### *Direct Exposure*

Direct exposure to MAP can occur through contact with either infected animals or people. Human to human transmission of MAP is not supported by epidemiologic evidence. There are no reports of family members of Crohn's disease patients having higher incidence of the disease or MAP exposure than the general population. The recent report of MAP culture from the breast milk of two Crohn's disease patients suggest a potential transmission route (Naser, Schwartz, & Shafran, 2000). However, a previous study indicates that Crohn's disease patients are less likely to have been breast-fed as infants than the general population (Bergstrand & Hellers, 1983).

Human MAP infection as a result of contact with infected animals, primarily cattle, is a more likely exposure route. To date, no study has found increased incidence of Crohn's disease among populations identified as having the greatest exposure risk—namely dairy farmers, veterinarians, and animal health diagnosticians having contact with MAP. Work by Cucino and Sonnenberg (2001) found that farmers had a significantly lower incidence of Crohn's disease than other occupational groups. A trend of higher incidence was noted in occupational groups not commonly associated with direct exposure to livestock, such as sales and administration positions. However, two studies have shown serological evidence of increased MAP infection rates among high exposure risk groups. Collins and Manning (1998) conducted serological testing utilizing a commercial bovine paratuberculosis ELISA test on a high exposure risk group (bovine veterinary practitioners), a moderate exposure risk group (Wisconsin farmers, not limited to dairy producers), and a low exposure risk group (urban blood donors). Results

indicated that 11.7 % of veterinarians sampled and 11.4 % of farmers sampled had been infected with MAP, as compared to 3.3 % of the low risk population. This result is significant in indicating both a higher infection risk for those in direct contact with MAP infected animals and providing evidence of MAP infection in the general population. An additional study (Chiodini & Thayer, 1997) found that animal disease diagnosticians working with MAP showed increased serological evidence of infection.

### *Environmental Exposure*

MAP shows enhanced longevity in the environment compared to most other *Mycobacteria* species (Whittington, Marshall, Nicholls, Marsh, & Reddacliff, 2004). The ability of MAP to survive in bovine fecal material has been reported for many years (Jorgensen, 1977) and recent work indicates that MAP is prevalent in environmental samples taken from infected dairy farms (Raizman et al., 2004). Of particular note, Raizman et al. (2004) demonstrated that MAP was occasionally present in water runoff from cow housing areas on infected farms. As previously mentioned, clustering of Crohn's disease cases has been documented in conjunction with bodies of water known to be at risk for fecal contamination from livestock operations. In the most recent study (Pickup et al., 2005), MAP was cultured from water samples taken from the Taff river above Cardiff, Wales, which has a significantly increased incidence of Crohn's disease. The study proposed that aerosolization of river water containing MAP may be an exposure factor. Van Kruiningen & Freda (2001) found that a cluster of Crohn's disease patients had all made recreational use of rivers and lakes in and around Mankato, MN. These bodies of water have a demonstrated history of contamination by fecal coliforms,

and have primarily agricultural drainage areas. Water contamination may also have played a role in the first documented Crohn's disease cluster in a rural English village (Allan, Pease, & Ibbotson, 1986). The source of the municipal water supply was subject to runoff from livestock grazing areas, and conventional water treatment processes utilizing chlorine are not always effective in eliminating MAP (Whan, Grant, Ball, Scott, & Rowe, 2001). In a study of familial clusters of Crohn's disease in Belgium (Van Kruiningen et al., 2005), the authors suggested that drinking well water as opposed to tap water was a significant risk factor for Crohn's disease. The estimated herd prevalence of Johne's disease in Belgium is 6 % (Boelaert et al., 2000), and it has been speculated that contamination of groundwater with MAP could account for this increased risk. These studies are suggestive of a link between environmental exposure to MAP and Crohn's disease.

#### *Dietary Exposure—Milk and Dairy Products*

The role of milk and dairy products in the transmission of MAP has received the most scientific attention of potential exposure risks. To date, there has been no association found between Crohn's disease and the consumption of milk. The Van Kruiningen et al. (2005) study is the first to suggest the consumption of unpasteurized cheese is a risk factor for Crohn's disease.

It has long been known that cows clinically infected with Johne's disease are capable of shedding MAP in their milk (Taylor, Wilks, & McQueen, 1981). More recent work has demonstrated that cows with subclinical Johne's disease are also capable of shedding the organism via milk (Sweeney, Whitlock, & Rosenberger, 1992). On-farm

fecal contamination of milk with MAP is also a possibility, as a 2004 study found substantial evidence of fecal contamination in bulk tank milk samples as demonstrated by the presence of fecal coliforms (Van Kessel, Karns, Gorski, McCluskey, & Perdue, 2004).

Regardless of route, it has been demonstrated that MAP is present in raw milk. Studies of bulk raw milk have shown that 12.9 % of Irish samples (O'Reilly et al., 2004), 19.7 % of Swiss samples (Corti & Stephan, 2002), and 7.8 % of United Kingdom samples (Grant, Ball, & Rowe, 2002) tested positive for MAP DNA utilizing PCR tests. A similar study of bulk raw milk has not been conducted in the United States, though PCR testing of milk tank samples from herds testing ELISA positive for MAP found 68 % prevalence of MAP DNA (Stabel, Wells, & Wagner, 2002). Culture of MAP from raw milk samples was reported in one of 389 samples that were PCR positive in the O'Reilly et al. (2004) study. These results indicate that raw milk could serve as an exposure source for human MAP infection if consumed without pasteurization.

Pasteurized milk has also been implicated as an exposure risk for MAP infections in people. Several studies have shown the potential for MAP to survive pasteurization in milk inoculated with the organism, with both dose and pasteurization process dependent response variability (Gao, Mutharia, Chen, Rahn, & Odumeru, 2002; Grant, Ball, Neill, & Rowe, 1996; Grant, Ball, & Rowe, 1998, 1999; Keswani & Frank, 1998; McDonald, O'Riley, Schroen, & Condrón, 2005; Pearce et al., 2001; Stabel & Lambertz, 2004; Stabel, Steadham, & Bolin, 1997; Sung & Collins, 1998). Generally, high doses of MAP inoculation were necessary to detect organism survivability as multiple log reductions in MAP detection were noted after various pasteurization procedures. Results of a study of

various pasteurization processes on milk naturally infected with MAP corroborated these findings (Grant, Hitchings, McCartney, Ferguson, & Rowe, 2002).

More indicative of actual exposure risk presented by pasteurized milk for MAP infection in people are studies of retail milk samples. A 1996 British study utilizing PCR to test pasteurized retail milk samples found 7 % of samples positive, with MAP detected in the macrophage-rich milk portions in 81 % of positive samples (Millar et al., 1996). Grant, Ball, and Rowe (2002) utilized PCR and culture to evaluate the presence of MAP in both raw and commercially pasteurized milk samples in the United Kingdom. MAP was detected by PCR in 11.8 % and cultured from 1.8 % of the pasteurized samples (rates by both methods in the raw samples were 7.8 % and 1.6 % respectively). Two Irish studies were unable to culture MAP from commercially pasteurized milk samples (O'Doherty, O'Grady, Smith, & Egan, 2002; O'Reilly et al., 2004), though O'Reilly et al. were able to detect MAP by PCR in 9.8 % of retail samples. In the Czech Republic, MAP was cultured from 1.6 % of retail commercially pasteurized milk samples (Ayele, Svastova, Roubal, Bartos, & Pavlik, 2005). The same study noted that 2 % of samples of locally pasteurized milk from known infected herds were culture positive. A recent study in the United States found that 2.8 % of 702 samples of retail pasteurized milk samples purchased in three states were both culture and PCR positive for MAP, with a seasonal trend of more positive samples in late summer and early fall (Ellingson et al., 2005). These studies indicate that pasteurized milk may serve as a primary milk-related exposure risk for MAP infections in people.

Other dairy products have been linked to human MAP exposure. As previously noted, the consumption of unpasteurized cheese has been postulated as a risk factor for

Crohn's disease. Experimental inoculation with MAP of raw milk used in the production of both hard and soft unpasteurized cheeses has been shown to allow MAP to survive throughout a 120 day ripening period (Spahr & Schafroth, 2001). Subpasteurization heat treatments used in cheese manufacture have been demonstrated to allow MAP survivability (Stabel & Lambertz, 2004). Laboratory inoculation of cheddar cheese prior to ripening has been shown to allow persistent though declining culturability of MAP over a 21 day period (Donaghy, Totton, & Rowe, 2004). In addition to pasteurization, salt and low pH are also used in cheese manufacture to reduce bacterial load. Though low pH is effective in reducing MAP survivability, salt concentration has no effect (Sung & Collins, 2000). The role of cheese in human MAP exposure should not be discounted, particularly as large portions of industrialized populations are more likely to consume unpasteurized cheese than unpasteurized liquid milk.

#### *Dietary Exposure—Meat*

The role of meat products as an exposure risk for MAP infections in people is little studied. There is no known association between the consumption of meat products and Crohn's disease, and no reports of MAP being cultured directly from bovine muscle tissue or retail beef products. There is evidence, however, that transmission through meat products may occur. Isolation of MAP from the blood and lymphoid tissue of cows with advanced Johne's disease has been demonstrated (Koenig et al., 1993). Chiodini and van Kruiningen (1986) found that 18 % of cull dairy cattle at slaughter in New England were positive for MAP based on histologic and bacteriologic examination of ileocecal valve, ileocecal lymph node, liver, tonsil, colon, and cecum tissues. Rossiter and Henning

(2001) reported similar results in isolation of MAP from thin sound market cows at slaughter. They isolated MAP from the feces or ileocecal lymph nodes of 34 % of dairy cattle and 3 % of beef cows sampled. MAP was isolated from the lymph nodes most closely associated with muscle used in ground beef, the superficial cervical and popliteal, from most of the cows with heavy fecal shedding and in 1.3 % of the overall cows sampled. This finding is significant, given approximately 17 % of ground beef comes from culled dairy cattle (Troutt & Osburn, 1997). Rossiter and Henning (2001) also corroborate studies indicating reduced exposure risk for non-dairy cattle, due to the lower prevalence of Johne's disease in beef cattle (Dargatz et al., 2001; Pence, Baldwin, & Black, 2003; Roussel et al., 2005) and age-related shedding and infection patterns (Kalis et al., 1999; Whitlock & Buergelt, 1996). More research is needed to fully evaluate the role of meat in MAP transmission to humans, especially as MAP is one of the most heat stable *Mycobacteria* species and may be resistant to currently recommended cooking temperature guidelines (Merkal, Crawford, & Whipple, 1979; Merkal & Whipple, 1980).

### **Strategies for Control of Human Exposure**

Each exposure route has unique possible control strategies, though evidence suggests that such measures are not foolproof. Precautions could be taken to limit the risk of infection via direct contact with infected animals, though the practicality of sufficient measures is questionable in dairy production settings. Water purification has not been shown to be 100 % effective in eliminating MAP from treated water, and does not address recreational or aerosol exposure to contaminated sources. Pasteurization of milk has not been demonstrated to completely safeguard milk or dairy products, and

exposure from unpasteurized product still remains a risk. Evidence suggests that MAP may be found in meat supplies, and consumer preparation of meat products may not be sufficient in eliminating risk.

Given the expense and difficulty of targeting specific exposure risks, control measures that address all are appropriate. Control of Johne's disease on the farm would be the most efficient critical control point prior to contamination of foods and the environment. There are four potential strategies for on-farm control of MAP. They are:

1. Test and cull programs. Though such programs have been largely successful in the eradication of other diseases such as tuberculosis and brucellosis, this is not considered a valid approach to control of Johne's disease due to the lack of sensitivity in the available tests, the expense, and lengthy time frame required (Groenendaal et al., 2002).
2. Treatment. Valid treatment programs for MAP infected animals are not available. Treatment protocols currently available prolong animal life but are not curative. Animals under treatment continue to shed the organism (St Jean & Jernigan, 1991).
3. Vaccination. Vaccination programs for Johne's disease do offer some potential in control of MAP, but they are subject to strict regulation and are not 100 % effective in preventing infection with and shedding of MAP (Larsen, Moyle, & Himes, 1978).
4. Management/Biosecurity. Management and biosecurity currently offers the best opportunity for MAP control on farms. The United States VJDHSP was designed to identify herds with good management and biosecurity practices that can

demonstrate negative screening results for Johne's disease (Bulaga, 1998). A primary goal of the program is to serve as a reservoir of low infection risk replacement animals for other producers, though the efficacy of this has not been demonstrated.

### **Objectives of the Study**

The primary objective of this project is to evaluate the infection risk for *Mycobacterium avium* subsp. *paratuberculosis* of replacement cattle purchased from VJDHSP herds and introduced into herds of unknown Johne's in order to determine the effectiveness of the program as a control strategy for reducing the prevalence of MAP in the food supply. The second objective of this project is to determine if producers enrolled in the VJDHSP perceive value to participation in order to market the program to non-enrolled dairy producers.

## **Method**

A list of the 26 Minnesota dairy producers at levels three or four of the Minnesota VJDHSP was obtained from the Minnesota Board of Animal Health (**MBAH**) in May 2003. In June and July 2003, up to five contact attempts via telephone were made to collect information regarding the sale of replacement cattle and the perceived benefits to participation in the VJDHSP. These questions covered the perceived economic advantages to VJDHSP participation, marketing of replacement cattle to other producers, and motivation for VJDHSP involvement. Sale records of replacement cattle sold by these herds were obtained, and four herds were identified as having purchased five or more heifers from VJDHSP herds and willing to participate in the next phase of the study.

The four producers participating in the second phase of the study had purchased cattle just prior to first calving from Level 3 or 4 VJDHSP herds, herein referred to as the VJDHSP birth cohort. For each VJDHSP birth cohort cow, three home-raised cows of the same lactation number (first, second, or third and higher) were randomly selected as controls, herein referred to as the control birth cohort. Control birth cohort selection was accomplished via systematic random sampling matched for lactation number with VJDHSP birth cohort cows. If sufficient control birth cohort cows were not available within a given lactation, cows were randomly selected from the previous if available or following lactation. If pre-selected control birth cohort cows were unavailable at the time of testing, another cow of the same lactation number was randomly selected as a replacement. Fecal samples were collected from the rectum of each cow using a disposable plastic sleeve. Blood samples were collected from VJDHSP birth cohort and

control birth cohort cows by caudal venipuncture. Body condition (1-5 scale, 1= excessively thin to 5=obese) and fecal (1-3 scale, 1= liquid consistency to 3=solid) scores were also evaluated.

Collected blood and fecal samples were submitted to the Minnesota Veterinary Diagnostic Laboratory for analysis. Blood collected was tested using a commercial serum ELISA test for antibodies to MAP (Collins & Manning, 1998). Fecal samples were tested by bacterial culture using Herrold's egg yolk medium and a 72 hour sedimentation procedure to concentrate the organism.

Results of diagnostic tests were analyzed both for the total population and within lactation number taking account of the matched case-control study design. Body condition (**BCS**) on a scale of 1-5 and fecal consistency (**FCS**) on a scale of 1-3 were summed as an indicator of clinical disease with any cow scoring three or lower out of a possible score of eight considered positive for clinical signs of Johne's disease. Relative risks to evaluate associations between cattle being reared in a VJDHSP versus an environment of unknown infection status and a positive test result for ELISA antibody detection, fecal culture status, and combined BCS/FCS were estimated by the Mantel-Haenszel procedure to account for the matched study design (Mantel & Haenszel, 1959). Relative risks, confidence intervals, and two sided Fisher's exact p-values were calculated using Stata (Statacorp LP, College Station, Texas) software.

## Results

Survey results for dairy producers currently enrolled at Level 3 or 4 of the MBAH VJDHSP are summarized in Table 1. Results indicated that 90% of enrolled producers perceived an economic advantage to participation in the VJDHSP, and that 95% felt that the advantages of the program outweighed associated costs. Reasons for participation in the VJDHSP varied, with the majority of producers (43%) citing herd health as the motivating factor. Other responses were marketability of surplus cattle (29%), free testing (19%), and consumer health (10%). A majority (71%) of respondents sold replacement cattle to other producers. Of these, 73% market cattle as being from a VJDHSP herd, 73% reported that VJDHSP status made replacement cattle easier to sell, and 53% received a VJDHSP status related premium.

At the time of survey, 15 (71%) of the respondents were selling replacement cattle. The remaining six were not selling cattle due to herd expansion. In total, 461 cattle were identified as having been sold from these herds since 1998. The mean number of cattle sold per producer was 21.1, the median was 18 with a range from zero to 65. All 15 producers selling replacement cattle reported that they were sold cattle as springing heifers. Seven of the 15 producers were able to report names of purchasers. Of the eight that could not, three sold their cattle internationally (Italy and China), three sold their cattle through a third party, and two felt that the information was confidential.

The use of VJDHSP status as a marketing tool was reported by 11 (73%) of the producers selling cattle. They indicated that there was a growing demand for their cattle and that presumed freedom from Johne's disease was important to their customers. This was corroborated by the fact that 73% of producers selling replacement cattle felt that

their VJDHSP status made selling replacement cattle easier (the remaining 27% reported that demand for replacement cattle was high enough that disease status was not a significant factor). Fifty-three % of those selling cattle did so at a premium. The mean premium received was \$190.63 with a range of \$50 to \$400. Test results for the four study herds purchasing replacement cattle from dairy producers enrolled at Level 3 or 4 of the MBAH VJDHSP are summarized by herd and lactation number for the total sample population in Table 2. Cows raised in infected herds were more likely to test positive to antibody to *M. paratuberculosis* using ELISA than cows raised in VJDHSP herds (relative risk = 8.67, 95% confidence interval = 1.20 to 62.5; p = 0.004). Of the 59 VJDHSP birth cohort cows, one cow was ELISA positive with a test result of 1.30. There were 26 ELISA positive control birth cohort cows with a mean test result of 1.17 (range 0.26 to 2.25). Though trends were observed, associations within lactation number were not statistically significant. Cows raised in infected herds were also more likely to shed detectable *M. paratuberculosis* using bacterial culture of feces than cows raised in VJDHSP herds, though this relationship was borderline statistically significant (p=0.046; relative risk = 2.33, 95 % confidence interval = 0.96 to 5.68). Of the 59 VJDHSP birth cohort cows, five cows were culture positive with a mean colony score of 1. There were 35 culture positive control birth cohort cows with a mean colony score of 1.54. Associations between fecal shedding and lactation number were not significant.

The association between birth cohort and combined BCS/FCS was not significant (relative risk = 3.67 (95 % confidence interval 0.48 to 27.80; p-value 0.30)).

## Discussion

The results of the survey of dairy producers currently enrolled at Level 3 or 4 in the MBAH VJDHSP indicate that there is economic value to participation in the program. Of the 21 producers interviewed, 90 % felt that they received an overall economic advantage through participation in the VJDHSP. Most reported that this benefit was related to increased productivity as a function of better cow health, but for several the dominant factor was premiums received on surplus cattle sold. Furthermore, 95 % of the respondents felt that the benefits of VJDHSP participation outweighed the management changes (i.e. calving management, manure disposal, testing regime) required to achieve and maintain their current status level in the program. Reasons for participation in the program varied, though many (43 %) did so out of a desire to improve herd health. Marketability of surplus animals was also a significant factor with 29 % of producers listing it as their primary interest in the program. The free testing offered by the MBAH at the inception of the program was the primary attraction for 19 % of producers surveyed. The remaining producers (9 %) listed concern over consumer health as their predominate reason for participation. The proposed link between Johne's disease in cattle and Crohn's disease was the foundation for this concern. Many of the other producers interviewed, though it was not their primary reason for participation, were also concerned about this potential link and the effect it could have on the dairy industry if substantiated. One advantage of the VJDHSP is providing a source of presumed low infection risk replacement cattle to the dairy industry.

The second phase of the study evaluated the assumption that purchasing replacement cattle from a VJDHSP herd reduced the likelihood of the purchased animals

developing Johne's disease. From the survey results listed above, four herds that had purchased five or more cattle from VJDHSP herds were willing to participate in the study. Study results indicated that Johne's disease was prevalent on all four farms. These herds had a total of 59 cattle (5, 14, 19, and 21 in each herd) in lactations one through four purchased from VJDHSP producers since 1998. Cows born and raised in infected herds were more likely to test positive using ELISA than cows born and raised in VJDHSP herds. The one VJDHSP birth cohort cow that tested ELISA positive was a second lactation cow that also tested positive for MAP in fecal culture. Of the 63 control birth cohort cows tested in this same herd, six were ELISA positive (four were also fecal culture positive). The remaining three herds tested yielded an additional 20 ELISA positive control birth cohort cows out of 177 tested (fourteen were fecal culture positive).

The association between birth cohort and ELISA test results was not statistically significant within individual lactation numbers, predominately due to small sample size. Of cows in lactation one, zero of the 21 VJDHSP birth cohort cows tested ELISA positive compared to nine of the 63 control birth cohort cows. For the second lactation, one of 22 VJDHSP birth cohort cows tested ELISA positive versus nine out of 66 control birth cohort cattle. For the third lactation and higher, zero out of 16 VJDHSP birth cohort cows were ELISA positive compared to eight out of 48 control birth cohort cows.

The association between birth cohort and fecal culture results for the entire sample population was borderline statistically significant. Of the 59 VJDHSP birth cohort cows, five were culture positive with a mean colony score of 1 (range 1), indicating less than 10 colonies per tube. There were 35 culture positive control birth cohort cows with a mean colony score of 1.54 (range 1 to 4), indicating a variable range

up to 100 or more colonies per tube. The positive VJDHSP birth cohort cows came from three of the four herds tested, and from various lactation numbers. Four of the five culture positive VJDHSP birth cohort cows and 15 of the 35 control birth cohort cows were ELISA negative, and these cows all received fecal culture scores of one indicating that they were light shedders. These cows were of various lactation numbers, with some being in their third lactation or higher. Given the high prevalence of Johne's disease on the sampled farms, environmental contamination with MAP is likely. It is also possible that, in heavily contaminated environments, cattle can ingest and passively shed MAP prior to development of infection and sufficient antibody to MAP.

Associations between birth cohort and fecal culture results were not significant by lactation number. For lactation one, one out of 21 VJDHSP birth cohort cows were fecal positive versus 14 out of 63 control birth cohort cows. For lactation two, three of 22 VJDHSP birth cohort and eight of 66 control birth cohort cows were fecal positive. For lactation three or higher, one of 16 VJDHSP birth cohort and 13 out of 48 control birth cohort cows were fecal positive.

FCS and BCS scores were summed as an indicator of clinical disease, as a cow with both a low body condition score and a loose fecal consistency would be indicative of possible clinical Johne's disease. Though diarrhea can precede loss of body condition in Johne's disease, disease is generally more apparent when both are present. There was little correlation between diagnostic test results and the combined BCS/FCS score (there was even less correlation when BCS and FCS were evaluated separately).

Study results indicate that cattle raised in a VJDHSP herd are less likely to be infected with MAP later in life. Results do not indicate that they are immune to

infection. The testing procedure required by the MBAH to reach Level 3 or 4 in the VJDHSP makes it highly unlikely that the VJDHSP birth cohort cows were infected with MAP prior to introduction to the sampled herds. Study data indicated that the farms sampled had high prevalence of Johne's disease and a heavy degree of environmental contamination, and infection with MAP for VJDHSP birth cohort cows after introduction based on our study results appears probable. However, results also indicate that, in heavily infected herds, purchase of replacement cattle from a VJDHSP herd does offer a lower infection risk for MAP than home reared cattle.

## CONCLUSION

Dairy farmers enrolled at Level 3 or 4 in the MBAH VJDHSP perceive an economic advantage in participating in the program, and report that program benefits outweigh necessary management changes. Furthermore, the VJDHSP appears to fulfill one of the stated goals of the program in providing low infection risk replacement cattle to other producers. This information will be of use in both promoting the VJDHSP, and ensuring its continuance.

Study results indicate that cattle reared in VJDHSP herds, even after residing in infected herds for variable time periods, are less likely to develop Johne's disease as measured by detectable antibody to MAP and by detectable fecal shedding. VJDHSP birth cohort cows also shed less MAP on a per-cow basis than cows born and raised in infected herds (less than 10 colonies per culture tube for all VJDHSP cattle, versus up to 100 or more colonies per culture tube for control birth cohort cattle). The high number of fecal culture positive cows that were ELISA negative is suggestive that passive shedding of MAP may occur in highly contaminated environments. Results did not indicate a significant difference in manifestation of clinical disease within birth cohorts as measured by body condition and fecal consistency scores

The results of this study suggest that cattle raised in VJDHSP herds are less likely to be infected with MAP leading to Johne's disease after introduction to infected herds than herdmates, demonstrating value of the VJDHSP as a control strategy for Johne's disease. Given the attention that the proposed link between Johne's disease in cattle and Crohn's disease in people continues to receive, and the continuing inability to either validate or disprove the association, this study is timely. Given the broad range of

potential exposure routes of humans to MAP, a farm based program offers the most comprehensive control strategy. Developing programs to reduce human exposure to MAP, and recruiting additional dairy producer participation, has real merit in preventing possible human infection.

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## TABLES

TABLE 1. Results of survey of dairy producers at level 3 or 4 of the MBAH VJDHSP

Question	Response	Response rate
Are you willing to participate in a survey regarding the VJDHSP? <sup>1</sup>	Yes	21 (100%)
	No	0 (0%)
Overall, do you realize an economic advantage by having a VJDHSP herd? <sup>1</sup>	Yes	19 (90%)
	No	2 (10%)
Do the benefits of participation in the VJDHSP outweigh necessary management changes? <sup>1</sup>	Yes	20 (95%)
	No	1 (5%)
What is your primary reason for participation in the VJDHSP? <sup>1</sup>	Herd Health	9 (43%)
	Marketability	6 (29%)
	Free Testing	4 (19%)
	Consumer Health	2 (10%)
Do you sell replacement cattle to other producers? <sup>1</sup>	Yes	15 (71%)
	No	6 (29%)
How many replacement cattle over 12 months of age have you sold since 1998? <sup>2</sup>	1 to 20	6 (40%)
	21 to 40	3 (20%)
	41 to 60	4 (27%)
	61 to 80	2 (13%)
Did these animals remain on your farm until point of sale? <sup>2</sup>	Yes	15 (100%)
	No	0 (0%)
Can you provide us with names of producerd who have purchased replacement cattle from you? <sup>2</sup>	Yes	7 (47%)
	No	8 (53%)
Do you market these cattle as being from a VJDHSP herd? <sup>2</sup>	Yes	11 (73%)
	No	4 (27%)
Does your VJDHSP status make replacement cattle easier to sell? <sup>2</sup>	Yes	11 (73%)
	No	4 (27%)
Do your replacement cattle sell at a premium? <sup>2</sup>	\$0	7 (47%)
	\$1 to 100	3 (20%)
	\$101 to 200	2 (13%)
	\$201 to 300	1 (7%)
	\$301 to 400	2 (13%)

<sup>1</sup>n = 21

<sup>2</sup>n = 15 (question was only asked of respondents selling replacement cattle)

TABLE 2. Results of diagnostic tests

	Number tested		ELISA positive		Fecal culture positive		BCS/FCS <sup>1</sup> positive	
	VJDHSP <sup>2</sup>	Control <sup>3</sup>	VJDHSP <sup>2</sup>	Control <sup>3</sup>	VJDHSP <sup>2</sup>	Control <sup>3</sup>	VJDHSP <sup>2</sup>	Control <sup>3</sup>
Total	59	177	1	26	5	35	1	11
Herd 1	19	57	0	7	1	9	0	2
Herd 2	5	15	0	6	0	6	0	2
Herd 3	14	42	0	7	2	10	1	1
Herd 4	21	63	1	6	2	10	0	6
1st lactation	21	63	0	9	1	14	0	4
2nd lactation	22	66	1	9	3	8	0	5
3rd/+ lactation	16	48	0	8	1	13	1	2

<sup>1</sup>Voluntary Johne's Disease Herd Status Program birth cohort

<sup>2</sup>Control birth cohort

<sup>3</sup>Combined body condition score/fecal consistency score

## APPENDICES

### A. Data collection instruments and letters

#### A.1. Survey of producers currently enrolled at level 3 or 4 of the Minnesota VJDHSP for cattle

1. The Minnesota Board of Animal Health and the College of Veterinary Medicine at the University of Minnesota are currently conducting a study on the usefulness of the Voluntary Johne's Disease Herd Status Program (VJDHSP) in providing low infection risk replacement animals to dairy producers. You have been contacted as a producer at level 3 or 4 in the program. Would you be willing to answer a few questions regarding your participation in the VJDHSP?
  - A. YES
  - B. NO
  
2. DO YOU OR DO YOU PLAN TO SELL REPLACEMENT CATTLE TO OTHER PRODUCERS?
  - A. YES
  - B. NO—GO TO QUESTION #9
  
3. HOW MANY REPLACEMENT CATTLE OVER 12 MONTHS OF AGE HAVE YOU SOLD SINCE 1998?
  - A. # \_\_\_\_\_
  - B. NONE—DO YOU PLAN TO SELL REPLACEMENT CATTLE OVER 12 MONTHS OF AGE IN THE NEXT FUTURE?
  
4. DID THESE CATTLE REMAIN ON YOU FARM UNTIL POINT OF SALE?
  - A. YES
  - B. NO—WHERE WERE THEY REARED?
  
5. We want to evaluate if replacement cattle purchased from herd in the VJDHSP remain free from the disease when exposed to it. Will you provide us with records of producers who have purchased replacement cattle from you?
  - A. YES—ENTER INFORMATION ON PAGE 2 AND RESUME SURVEY
  - B. NO
  
6. Do you market these cattle as being from a VJDHSP herd?
  - A. YES
  - B. NO
  
7. Does your VJDHSP status make replacement cattle easier to sell?
  - A. YES
  - B. NO

8. Do your replacement cattle sell at a premium?
- A. YES—**AMOUNT PER HEAD \$**\_\_\_\_\_
  - B. NO
9. Overall, would you say you realize an economic advantage by having a herd free of Johne's disease?
- A. YES
  - B. NO
10. Do the benefits of participation in the VJDHSP outweigh management changes required to maintain status?
- A. YES
  - B. NO
11. What is your primary reason for participating in the VJDHSP (i.e. marketing, future plans, salability of cattle)?

A.2. Sample collection consent form

Herd owner consent form for study

‘Evaluation of Susceptibility of Adult Dairy Cattle to Infection with M.  
Paratuberculosis’

The Minnesota Board of Animal Health and the College of Veterinary Medicine at the University of Minnesota is conducting a study to evaluate the susceptibility of adult animals to the agent causing Johne’s disease. Dairy cattle reared in an environment presumed free of the agent will be sampled to determine if they are resistant when exposed as adults. Laboratory testing of fecal and blood samples for evidence of or exposure to the agent causing Johne’s disease will be used as the critical outcome measure evaluated. This information will be used to evaluate methods of disease control on commercial dairy operations.

I, \_\_\_\_\_,  
agree to participate in this study, conducted by the Minnesota Board of Animal Health and the College of Veterinary Medicine at the University of Minnesota. As part of this study, I agree to allow blood and fecal sampling of cattle in my herd. I recognize that risks to cattle associated with collection of blood and fecal samples of cattle are minimal. I also recognize that my participation in this project is voluntary, and that I can withdraw from the study at any time.

Sincerely,

CLIENT NAME \_\_\_\_\_ DATE \_\_\_\_\_

PRINCIPAL INVESTIGATOR NAME (contact person if questions or problems arise)

\_\_\_\_\_ DATE \_\_\_\_\_

Scott J. Wells 612-625-8166

University of Minnesota Institutional Animal Care and Use Committee 612-626-5654